

SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Common (Usual) Name:	Qualitative Colorectal Cancer Screening Test
Trade (Proprietary) Name:	Epi proColon
Applicant's Name and Address:	Epigenomics AG Kleine Präsidentenstrasse 1 Berlin, Germany 10178
Date of Panel Recommendation:	TBD
Premarket Approval Application (PMA) Number:	P130001
Date of FDA Notice of Approval:	TBD
Expedited:	Not Applicable

II. INDICATIONS FOR USE

The Epi proColon test is a qualitative *in vitro* diagnostic test for the detection of methylated Septin9 DNA in EDTA plasma derived from patient whole blood specimens. Methylation of the target DNA sequence in the promoter region of the *SEPT9_v2* transcript has been associated with the occurrence of colorectal cancer (CRC). The test uses a real-time polymerase chain reaction (PCR) with a fluorescent hydrolysis probe for the methylation specific detection of the Septin9 DNA target.

The test is indicated to screen patients for colorectal cancer who are defined as average risk for colorectal cancer (CRC) by current CRC screening guidelines. Patients with a positive Epi proColon test result should be referred for diagnostic colonoscopy. Men and women 50 to 85 years of age were included in the Epi proColon clinical trial. The Epi proColon test results, together with the physician's assessment of history, other risk factors, and professional guidelines, may be used to guide patient management.

The Epi proColon test is for use with the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument.

III. DEVICE DESCRIPTION

The Epi proColon test is an *in vitro* polymerase chain reaction (PCR) assay for the qualitative detection of methylated Septin9 DNA isolated from 3.5 mL of patient plasma. Cytosine residues in the v2 region of the Septin9 gene become methylated in colorectal cancer (CRC) tissue but not in normal colon mucosa. This aberrant methylation can be detected by specific amplification of Septin9 DNA present in the plasma sample. Detection of CRC DNA in plasma using the methylated Septin9 DNA biomarker has been demonstrated in multiple case control studies of CRC patients and colonoscopy-verified negative controls (deVos T., 2009) (Lofton-Day

C., 2008) (Gruetzmann R., 2008) (Model F., 2007) as well as in two multicenter clinical evaluations. The blood-based Epi proColon test offers patients who do not undergo screening by other recommended methods an alternative option to participate in a CRC screening program.

The Epi proColon test includes three components:

- 1) The Epi proColon Plasma Quick Kit (M5-02-001) for purification and bisulfite conversion of DNA from K₂EDTA plasma.
- 2) The Epi proColon Sensitive PCR Kit (M5-02-002) for duplex PCR amplification of the methylated Septin9 target and an ACTB (β -actin) internal control.
- 3) The Epi proColon Control Kit (M5-02-003) contains Positive Controls and Negative Controls for monitoring the successful execution of the workflow and for ensuring validity of test results.

Materials provided with the Epi proColon Plasma Quick Kit (M5-02-001)

The contents of the Epi proColon Plasma Quick Kit are listed in Table 1. The kit is shipped at ambient temperature and stored at room temperature (15°C to 30°C).

Table 1: Contents of the Epi proColon Plasma Quick Kit

Item	Reagent	Reagent Components	Containers	Nominal Volume	Used For
1	Epi proColon Lysis Binding Buffer	Guanidinium thiocyanate	1 bottle	125 ml	DNA extraction
		Triton X-100			
2	Epi proColon Magnetic Beads	Dynabeads® Magnetic Particles	1 bottle	4 ml	DNA extraction & purification
3	Epi proColon Wash A Concentrate	Guanidinium thiocyanate	1 bottle	60 ml	DNA extraction & purification
		Triton X-100			
4	Epi proColon Elution Buffer	Tris buffer	1 tube	6 ml	DNA extraction & purification
5	Epi proColon Bisulfite Solution	Ammonium bisulfite solution	4 tubes	1.9 ml each	Bisulfite conversion
6	Epi proColon Protection Buffer	THFA	1 tube	1 ml	Bisulfite conversion
		Trolox			
7	Epi proColon Wash B Concentrate	Molecular biology grade water	1 bottle	7 ml	DNA extraction & purification

Materials provided with the Epi proColon Sensitive PCR Kit (M5-02-002)

The contents of the Epi proColon Sensitive PCR Kit are listed in Table 2. The kit must be shipped frozen and stored at -25°C to -15°C.

Table 2: Contents of the Epi proColon Sensitive PCR Kit

Item	Reagent	Reagent Components	Containers	Nominal Volume
1	Epi proColon PCR Mix	10X PCR buffer	2 tubes	810 µl each
		MgCl ₂		
		Nucleotide Mix with dUTP		
		Oligonucleotides		
2	Epi proColon Polymerase	Taq DNA Polymerase	1 tube	85 µl

Materials provided with the Epi proColon Control Kit (M5-02-003)

The Epi proColon Control Kit contains sufficient Positive and Negative Control vials for performing six independent runs (Table 3). The Epi proColon Control Kit must be shipped frozen and stored at -25°C to -15°C.

Table 3: Contents of the Epi proColon Control Kit

Item	Reagent	Reagent Components	Containers	Nominal Volume
1	Epi proColon Positive Control	Tris-EDTA buffer	6 tubes	3.6 ml each
		Bovine serum albumin Fraction V		
		Jurkat cancer cell line DNA		
		HeLa cancer cell line DNA		
2	Epi proColon Negative Control	Tris-EDTA buffer	6 tubes	3.6 ml each
		Bovine serum albumin Fraction V		
		Jurkat cancer cell line DNA		

Real-Time PCR Instrument and Software

The Epi proColon test has been validated for use with the Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument using the accessories recommended by the instrument manufacturer. The installation, calibration, performance verification and maintenance of the Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument must be performed according to the instrument manufacturer's instructions. The Epi proColon test was validated with Sequence Detection Software v1.4 - 21 CFR Part 11 Module. The Sequence Detection Software v1.4 is utilized as off-the-shelf and no other software is required to analyze or interpret the test results.

Materials Required, But Not Provided with the Kit

In addition to materials provided with the kit, standard consumables, reagents and equipment available in molecular diagnostics laboratories are needed to perform the test. Materials required, but not provided by Epigenomics are listed in the Epi proColon Instructions for Use.

Properties of the Device Related to Detection of Colorectal Cancer

The Epi proColon test is predicated on the observation that cancer specific DNA can be detected in the blood of individuals with colorectal cancer. It is known that DNA from tumor cells can enter the blood stream through necrosis or apoptosis of the malignant cells. The presence of free circulating tumor DNA in blood was first described in the 1930's and first associated with the presence of cancer in the 1970's. With the development of sensitive detection methods, the tumor origin of DNA in plasma of cancer patients was substantiated by PCR amplification of genetic mutations occurring in the tumor tissue. In the ensuing years, hundreds of published studies corroborated the general observation that tumor DNA can be detected in serum or plasma of cancer patients. Increased methylation in the promoter region of genes is well-characterized in tumor biology, and extensively described for colorectal cancer (CRC). The Epi proColon test identifies a region of the v2 promoter of the Septin9 gene that is highly methylated in colorectal cancer tissue and which has been shown to be detectable in plasma of individuals with colorectal cancer.

Principles of Operation

Specimen Preparation

Approximately 10 mL patient blood is collected using an EDTA coated blood collection tube (Vacutainer® K₂ EDTA Tube, Becton Dickinson). Plasma is isolated as described in the Epi proColon instructions for use (IFU). An additional centrifugation is performed to remove any remaining cellular material. The resulting plasma is stored frozen at -25°C to -15°C or processed immediately with the Epi proColon test.

Conducting DNA Extraction, Bisulfite Conversion, and DNA Purification

The Epi proColon test is designed for laboratory professionals familiar with DNA extraction methods and real-time PCR. The procedure begins with the extraction of DNA contained in 3.5 ml of patient plasma. The DNA is bound to magnetic beads during an incubation step, and then magnetically separated from the plasma. Impurities are removed from the magnetic beads in a wash step. Purified DNA is removed from the magnetic beads by eluting the DNA with elution buffer.

The eluate containing DNA is then subject to a chemical reaction (bisulfite treatment) that specifically modifies unmethylated cytosine residues within the purified DNA. After several washing steps, the bisulfite converted DNA is eluted with 60 µl Epi proColon Elution Buffer to yield about 55 µl final eluate volume.

Conducting Real-time PCR

Real-time PCR is conducted in three replicates per sample with 15 µl bisulfite converted DNA, each. Thus, 45 µl of the 55 µl eluate per sample are used for analysis. The sample DNA is added

to a mixture of Epi proColon PCR Mix and Epi proColon Polymerase for a final volume of 30 µl per replicate. The reaction mixtures are amplified using the Applied Biosystems® 7500 Fast Dx Real-Time PCR instrument.

The Epi proColon test detects the Septin9 and ACTB signals utilizing two independent fluorescence channels of the Applied Biosystems® 7500 Fast Dx Real-Time PCR instrument. Specific analysis settings are defined in the Epi proColon IFU and must be applied to analyze the PCR results.

Workflow Controls Provided by Epigenomics

The Epi proColon Control Kit (M5-02-003) contains Epi proColon Positive and Negative Controls. These controls are processed in each run of patient specimens to monitor the successful execution of the procedure and to ensure validity of test results. The Septin9 and ACTB values for the Epi proColon Positive and Negative Controls must be within the limits specified in the Epi proColon IFU. If control results are out of their specified limits, the associated patient specimen test results are invalid, must not be reported, and the specimen must be retested.

Internal Control

The detection of ACTB DNA in the real-time PCR reaction serves as an internal control for each patient specimen. The control monitors for adequate specimen quality and specimen preparation. The result of an individual Septin9 PCR replicate is invalid when Ct values of the ACTB PCR are > 32.1. High ACTB Ct values indicate low DNA content in the respective specimen or an inadequate DNA preparation.

Results Interpretation

The interpretation of patient results is performed manually and requires valid Epi proColon Positive and Negative Controls in the respective PCR run. An interpretation worksheet is available with the IFU. The Epi proColon Positive Control is valid when all three PCR replicates result in Ct values ≤ 29.8 for ACTB and in Ct values ≤ 41.1 for Septin9. The Epi proColon Negative Control is valid when all three PCR replicates result in Ct values ≤ 37.2 for ACTB and all replicates are negative for Septin9.

Once a PCR run has been determined to be valid based on the control results, patient specimen results are evaluated. When the result of the ACTB internal control in a single PCR replicate is ≤ 32.1 Ct, the PCR reaction is valid and the Septin9 signal in that reaction can be analyzed.

The Epi proColon test result for a patient specimen is interpreted “POSITIVE” when at least one of the three PCR replicates has both a valid ACTB result and a positive Septin9 PCR result. A Septin9 result is positive when a Ct value is reported in the PCR run. The Epi proColon test result for a patient specimen is interpreted “NEGATIVE”, when all three PCR replicates are valid (ACTB ≤ 32.1 Ct) and all three Septin9 PCRs are negative.

IV. PRECAUTIONS, WARNINGS AND LIMITATIONS

Patient Precautions

- Detection of colorectal cancer is dependent on the amount of free circulating tumor DNA in the specimen and may be affected by sample collection methods, sample storage, patient factors and tumor stage.
- The Epi proColon test is an alternative screening method for patients who are defined as average risk for colorectal cancer by current screening guidelines, and who are unwilling, unable or do not undergo screening by other recommended screening methods.
- The Epi proColon test has not been evaluated in persons:
 - Considered to be at higher risk for developing colorectal cancer, or with a previous history of colorectal polyps or colorectal cancer. Persons at higher risk include those with a family history of colorectal cancer, particularly with two or more first-degree relatives with colorectal cancer, or one or more first degree relative(s) less than 50 years of age with colorectal cancer.
 - With known hereditary non-polyposis colorectal cancer (HNPCC) or familial adenomatous polyposis (FAP).
 - With anorectal bleeding, hematochezia, or with known iron deficiency anemia.
- There is insufficient evidence to report programmatic sensitivity of the Epi proColon test over an established period of time.
- CRC Screening guideline recommendations vary for persons over the age of 75. The decision to screen persons over the age of 75 should be made on an individualized basis in consultation with a healthcare provider.
- The Epi proColon test demonstrated non-inferiority to a FIT test (OC FIT-CHEK® Polymedco), for sensitivity but not for specificity, indicating that the Epi proColon test exhibited a higher rate of false positive results compared to the FIT test.
- Test results should be interpreted by a healthcare professional.

Laboratory Precautions Related to Real-Time PCR

- The Epi proColon test is for *in vitro* diagnostic use only
- This procedure is for professional laboratory use only and assumes familiarity with DNA extraction methods and real time PCR assays
- Compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples during and after the DNA extraction, bisulfite conversion, and purification procedure
- Use only single-use pipettes and filter tips to prevent cross-contamination of the patient sample
- Use of reference pipettes for pipetting extracted and bisulfite treated DNA is strongly recommended

- Good technique is important to prevent the introduction of nucleases into samples during the extraction procedure
- Do not freeze extracted DNA
- Epi proColon bisulfite solution is sensitive to oxygen contact; use only unopened tubes of Epi proColon Bisulfite Solution; do not store but discard any left-over solution
- When removing liquid from microtubes in multiple steps in the procedure, take care not to remove magnetic beads
- Strict separation of pre-PCR activities (e.g., plasma DNA extraction and purification, PCR setup) and post-PCR activities (e.g., real-time PCR) is highly recommended to prevent contamination by amplicons generated from previous PCR testing
- To prevent the release of any PCR product, used PCR plates should be placed in a resealable plastic bag immediately after removal from the PCR instrument, and the bag closed and disposed of in a dedicated PCR waste container
- Never open a used PCR plate or store a used PCR plate outside of the PCR instrument

Additional Precautions

- Do not mix kit components between kit lots
- Do not use kits or kit components beyond their stated expiration date
- Do not freeze whole blood K2EDTA blood or blood tubes
- The Epi proColon test kits do not contain infectious substances or agents that may cause disease in humans or animals
- All patient blood and plasma specimens should be handled as though they are capable of transmitting disease. Observe universal precautions and safe laboratory procedures as specified in the OSHA Standard on Bloodborne Pathogens, CLSI Document M29-A3, and any other appropriate biosafety practices as required by your laboratory.

Warnings

- The Epi proColon test is not intended to replace colorectal screening by colonoscopy.
- The Epi proColon test is not intended to screen persons under the age 50 who are considered to be at average risk for colorectal cancer.
- Positive Epi proColon test results are not confirmatory evidence for the presence of colorectal cancer. Patients with a positive Epi proColon test result should be referred for diagnostic colonoscopy.
- A negative Epi proColon test result does not guarantee absence of cancer. Patients with a negative Epi proColon test result should be advised to continue participating in a colorectal cancer screening program that also includes colonoscopy, fecal tests and/or other recommended screening methods.

- Positive test results have been observed in clinically diagnosed patients with chronic gastritis, lung cancer and also in pregnant women^{1,2}.

Limitations

- This product has been validated for the combination of the Epi proColon Plasma Quick Kit (M5-02-001), the Epi proColon Sensitive PCR Kit (M5-02-002), and the Epi proColon Control Kit (M5-02-003) only. These kits and components (DNA extraction, bisulfite conversion or PCR kits) are not interchangeable or replaceable with other manufacturer's products.
- The Epi proColon test has been validated for use only with plasma derived from blood collected with BD Vacutainer® K₂ EDTA blood collection tubes (Becton Dickinson). Do not use this test with other clinical specimen types or with other blood collection tubes.
- The Epi proColon test has been validated for use only with the Applied Biosystems® 7500 Fast Dx Real-Time PCR Instruments with Sequence Detection Software v1.4 21 CFR Part 11 Module. Do not use with other instruments or software.
- Use of this test is limited to personnel experienced and trained in performing PCR assays. Good laboratory technique is essential and failure to follow instructions provided in these instructions may produce erroneous results.

V. ALTERNATIVE PRACTICES AND PROCEDURES

There are a variety of alternative practices and procedures for colorectal cancer (CRC screening) that may be divided into invasive and non-invasive methods. Established invasive methods comprise colonoscopic and sigmoidoscopic examination followed by a pathological diagnosis, while established non-invasive methods include fecal occult blood testing (FOBT) by either guaiac-based (gFOBT) or immunochemical-based (fecal immunochemical test, FIT) detection.

Colonoscopy for average-risk men and women ages 50 years and older is recommended every ten years in countries or healthcare settings with sufficient endoscopic, financial and professional resources. Colonoscopy followed by histopathological evaluation reduces CRC incidence by 50-76% and CRC mortality by 30-65% (Winawer SJ, 2011). CRC screening by colonoscopy is regarded as the gold standard, as it offers both early stage cancer detection and cancer prevention by removal of pre-cancerous lesions (Rex DK, 2009). Unfortunately, the procedure is not well accepted by the target population, as indicated by data from Germany where it has been systematically evaluated for over eight years. In the German setting, only 2.6% of the target population has participated in colonoscopy screening, despite its affordability (offered free of charge) and its support by national media campaigns (Brenner H., 2010). Recent studies have also indicated that the CRC incidence (Baxter N., 2011) (Brenner H.,

1 Warren, D. et al. Septin 9 methylated DNA is a sensitive and specific blood test for colorectal cancer. BMC Medicine, 9:133 (2011)

2 Epigenomics data on file.

2010) and mortality (Singh H., 2010) reductions may be limited to the rectum and the left side of the colon.

Flexible sigmoidoscopy (FS) is recommended in settings where endoscopy resources are limited and FS resources are available. It is recommended in average-risk men and women ages 50 years and older every five years. For a positive FS, which means that either an advanced adenoma or an adenoma of any type is found depending on the health care system, a diagnostic work-up with full colonoscopy is recommended (Winawer S., 2011). However, there is no solid evidence that the presence or absence of distal adenomas coincides with the presence or absence of proximal cancers or pre-cancerous lesions.

In settings where both colonoscopy and FS are limited, FOBT (either gFOBT or FIT) is recommended annually beginning at age 50, with a preference for high-sensitivity tests (Winawer S., 2011). According to the US Multi-Society Task Force on CRC screening (Levin B., 2008), sensitivity of gFOBT is highly variable as compared to the gold standard of colonoscopy. In a study of 10,702 eligible patients (Allison J., 1996), sensitivity for CRC ranged between 37.1 and 79.4%. By contrast, Imperiale et al. (Imperiale T., 2004) reported a sensitivity of only 12.9% for CRC. In a more recent study, Allison et al. 2007 (Allison J., 2007) reported a sensitivity of 64.3% using the high sensitivity gFOBT Hemoccult Sensa (95% CI 35.6 - 86.0%). Park et al. (Park DI, 2010) reported gFOBT sensitivity at 30.8% (95% CI 9.0 - 61.4%) in an average risk Korean population of 770 patients. The Multi-Society CRC screening guideline (Levin B., 2008) observed no clear pattern of superior performance for a high-sensitivity gFOBT (Hemoccult Sensa) versus a variety of FITs. Allison et al. (Allison J., 2007), reported FIT sensitivity for CRC at 81.8% (95% CI 47.8 - 96.8%). Smith et al. (Smith, 2006) observed a FIT sensitivity of 82% in 17 CRC cases. Morikawa et al. (Morikawa T., 2005) conducted a prospective comparison of 1-time FIT to colonoscopy in a population of 21,085 asymptomatic adults in which 79 CRCs (0.4%) were diagnosed. The sensitivity of 1-time FIT for CRC was 65.8% (95% CI 55.4 – 76.3%). Park et al. (Park D., 2010) reported FIT sensitivities between 84.6% and 92.3% (95% CI 54.6 - 98.1%, depending on the choice of hemoglobin thresholds).

In addition to the established endoscopy and FOBT tests, new technologies have become available which are not yet part of medical routine practice including computed tomography colonography (CTC), capsule endoscopy and stool DNA testing.

CTC is based on constructing two- and three-dimensional images to investigate the presence of cancerous and pre-cancerous lesions in colon and rectum. In a recent review, Whitlock et al. (Whitlock E., 2008) concluded that CTC is not ready for routine use. One of the greatest concerns raised was the lack of data on the potential long-term health consequences of radiation used in CTC.

With capsule endoscopy, a pill-sized camera is swallowed to visualize the gastrointestinal tract. In recent studies (Eliakim R., 2009) (Gay G., 2009) (Sieg A., 2009) (Van Gossum A., 2009), the range of sensitivity reported was between 72 and 78%. Capsule endoscopy requires bowel preparation similar to colonoscopy and may share issues of acceptance at an inferior performance.

Stool DNA testing investigates the presence of CRC-specific methylated DNA or DNA mutations in stool. The draft Comparative Effectiveness Review prepared for the Agency for Healthcare Research and Quality concluded that fecal DNA tests have insufficient evidence supporting their diagnostic accuracy to screen for CRC in asymptomatic average-risk patients (Lin J., 2012).

There are no other *in vitro* diagnostic tests targeting Septin9 methylation and no other blood based screening test for CRC approved for use in the U.S.

VI. MARKETING HISTORY

Epigenomics has not marketed the Epi proColon test in the United States. Epigenomics has marketed the Epi proColon test as an *in vitro* diagnostic (IVD) product in Europe in the form of a CE marked blood plasma-based test for methylated Septin9 DNA (mSEPT9). This test has been marketed in Europe under the brand name Epi proColon since September 2009. A second generation test has been available in Europe under the brand name Epi proColon 2.0 CE since February 2012.

Epi proColon 2.0 CE is currently sold in the European Union and the Asian Pacific region.

VII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

The risk associated with the Epi proColon test is the occurrence of a false negative result. A false negative result would delay follow-up procedures that would diagnose colorectal cancer, such as colonoscopy. To address the potential of a false negative finding, a regular screening program should be recommended to patients and clinical symptoms and other risk factors should be followed up accordingly. Comparative results indicate that the risk posed by a false negative test result is similar to the risk posed by other non-invasive screening methods such as FIT.

There is also the risk of a false positive result with the Epi proColon test. To address this risk, patients with a positive Epi proColon test result should be referred for diagnostic colonoscopy. Users are warned in the IFU that a positive Epi proColon result is not confirmatory evidence for the presence of colorectal cancer.

VIII. SUMMARY OF PRECLINICAL STUDIES

A. Laboratory Studies

1. Comparison to Reference Method

To verify the accuracy of the Epi proColon test compared to colonoscopy, an internal accuracy study was conducted with 346 clinical samples and presented with an overall clinical sensitivity and specificity of 94.9% (95% CI 88.6 – 97.8%), and 84.3% (79.2 – 88.3%) respectively.

Colorectal cancer samples were collected at European centers in a case/control setting where blood was drawn after the disease was confirmed by colonoscopy. The samples were designated as either positive for colorectal cancer (CRC) or negative (normal). The data generated in this study were used to estimate clinical performance of the assay with respect to identification of CRC.

Table 4: Summary of Accuracy Data for Epi proColon Assay Combined Data

Epi proColon Assay	Colonoscopy		Total
	Positive (CRC)	Negative (NED)	
Positive	93	39	132
Negative	5	209	214
Total	98	248	346

2. Analytical Sensitivity -Limit of Detection

The Limit of detection (LoD) of the Epi proColon test was determined at four laboratories according to CLSI EP12 and EP17 guidance documents. All sites utilized the test kit manufactured under final manufacturing conditions. Seven levels of technical samples were tested with a range of HeLa cancer cell line DNA from 0 (blank) to 50 pg/mL. LoDs were determined using DNA spiked into plasma and into an artificial matrix of Tris buffer plus BSA. Results were analyzed for positivity based on the instructions for use provided with the test. The LoD was calculated for all samples combined (both artificial matrix and plasma) as well as only for samples spiked in plasma. The LoD for samples spiked in plasma was 4.7 pg/mL with 95% CI (2.5-9.0 pg/mL) as illustrated in Figure 1. The observed difference between LoD estimates of 4.7 pg/mL for human plasma and 8.0 pg/mL for buffer matrix corresponds to a single haploid genome per ml, a difference that is not biologically significant.

For the purpose of the test kit, we report the LoD of 4.7 pg/mL, based on test material spiked in a plasma background.

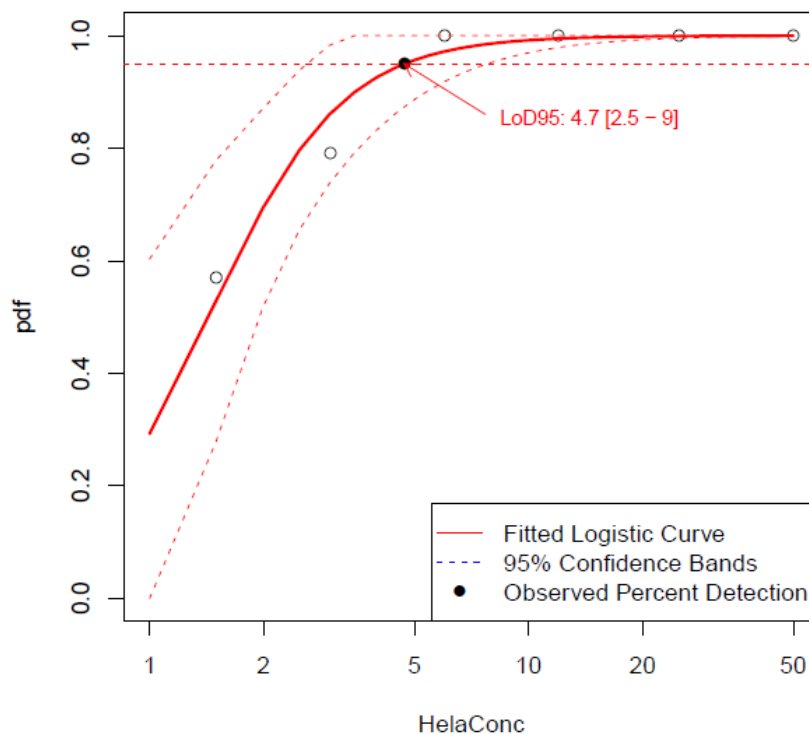


Figure 1. Observed positive detection fraction (pdf) as a function of Septin9 concentration (log scale). The fitted logistic regression model is represented in red with dashed red lines indicating the 95% confidence interval.

3. Precision

Studies to evaluate repeatability and intermediate precision of the assay were performed internally, and repeated along with an assessment of reproducibility in external laboratories. Table 5 summarizes the parameters for the studies.

Table 5: Precision Parameters

Parameters	Internal Site	External Sites
Samples	9 clinical pools: 6 from CRC patients 3 from healthy patients	14 clinical pools: 6 from CRC patients 3 from healthy patients 5 from CRC spikes into plasma
Number of Sites	1	3
Number of Operators	3	6
Number of Reagent Lots	3	3*
Number of Instruments	2	3

*Controls evaluated with single lot at each site.

Repeatability and Intermediate Precision

To evaluate repeatability and intermediate precision of the assay, aliquots from 14 clinical sample pools were tested at three testing sites by six operators with three reagent lots using three PCR instruments. Each plasma pool was tested 12 times. Pools 1 – 6 generated from CRC plasma were positive in all 12 tests. For the three pools representing self-declared healthy blood donors, pool 7, pools 8 and pool 9 were each negative in 9 out of 12 tests. For the pools derived by diluting a single CRC plasma aliquot in human plasma, pools 10, 13, and 14 were positive in 11 out of 12 tests, while pools 11 and 12 were positive in all 12 replicates. In total, for 129 out of 132 samples where CRC plasma was tested (pools 1 – 6, pools 10 – 14), the test result was positive leading to 98% (94 – 99%) positive percent agreement with clinical status. Aggregated over the three pools derived from healthy blood donors (pools 7 – 9), the test results for 27 out of 36 samples were negative, leading to 75% (59 – 86%) negative percent agreement with clinical status. The total percent agreement estimated from these data is 156/168, i.e. 93% (88 – 96%). There were no differences in the positive and negative percent agreement attributable to sites, operators or kit lots.

In addition, precision and reproducibility analyses were conducted with Ct values generated on the set of 14 sample pools. For precision, the ranges of standard deviation and coefficient of variation for Septin9 were 0.4 – 2.1 Ct and 1.1 – 5.5%, respectively. The corresponding ranges for ACTB were 0.2 – 0.4 Ct and 0.8 – 1.7%.

For reproducibility, the ranges of standard deviation and coefficient of variation for Septin9 were 0.4 – 2.3 Ct and 1.4 – 6.0%, respectively. The corresponding ranges for ACTB were 0.2 – 0.4 Ct and 0.7 – 1.6%.

4. Robustness

a. Assay Controls and Failure Modes

Robustness of the test and effectiveness of the assay's control system were initially investigated internally through analysis of potential failure modes. An extensive analysis was performed for failures that could occur at each step of the workflow process.

Each of the twenty (20) failure modes were tested with four different technical samples: 1 analyte negative plasma specimen, 1 analyte positive plasma specimen, 1 Positive Control, and 1 Negative Control (from the Epi proColon Control Kit).

After the evaluation of all failure modes it was found that the assay control system was successful in identifying situations in which incorrect test measurements might occur. In these critical failure situations either cycle threshold cutoffs or assay QC limits were not met and samples were therefore deemed invalid, preventing reporting of a potentially incorrect value.

b. Bisulfite Conversion

In order to verify the assay specificity and functionality of the control system for bisulfite conversion, incomplete conversion was induced by reaction conditions outside of the specifications.

These conditions were performed on four types of technical samples: 1 analyte negative plasma specimen, 1 analyte positive plasma specimen, 1 Positive Control, and 1 Negative Control (from the Epi proColon Control Kit).

None of the conditions tested caused false positive /negative results, since the control system identified relevant failure modes.

The following conditions were observed to be important and are noted in the instructions for use.

- Deviations from the specified amount of beads may lead to false results. Users are warned to ensure correct magnetic bead concentration by mixing the bead suspension thoroughly just before pipetting.
- If the extracted DNA is not used immediately, it can be stored at 2 to 8°C for up to 24 hours. However, loss of activity was noted for extracted DNA that was frozen prior to bisulfite conversion. The user is instructed to not freeze the extracted DNA prior to bisulfite conversion.
- Epi proColon Bisulfite Solution is sensitive to oxygen contact. The user is instructed to use only unopened tubes of Epi proColon Bisulfite Solution and to discard any left-over solution.
- Over-drying was found to reduce bisDNA recovery. The user is instructed to only use the specified drying time and temperature.

5. Analytical Specificity - Interfering Substances

A study was conducted to verify that the presence of interfering substances potentially found in plasma samples have no effect on the test results. The ten (10) most common substances present in human plasma were selected and tested at the highest concentration that would occur in a clinical setting.

Interference was not observed when the substances were tested at the following concentrations: albumin (26 mg/mL), bilirubin (0.2 mg/mL), cholesterol (5 mg/mL), glucose (10 mg/mL), hemoglobin (10 mg/mL), triglycerides (12 mg/mL), K2EDTA (20 mg/mL), red blood cells (0.26% v/v), uric acid (0.235 mg/mL) and human sperm DNA (66 ng/mg). Positive results were detected when three substances were tested at higher concentrations: albumin (40 mg/mL), red blood cells (0.4% v/v) and human sperm DNA (100 ng/mL).

6. Cross Reactivity

a. Blast Search Of Primers

An Ensembl Genome Browser-Blast search was performed for the amplicon region of methylated Septin9. No repeat sequences were found in this region. The NCBI database was also searched for the existence of single nucleotide polymorphisms (SNPs) in the Septin9 amplicon region. One C/T variation was found at rs7217986. This polymorphism does not affect the amplification of the expected product since the C-variant is converted

to uracil during bisulfite treatment which is then incorporated as the base thymine (T) during the PCR reaction.

An Ensembl Genome Browser-Blast search was performed for the amplicon region of ACTB. Three SNPs were identified in the region of the ACTB amplicon. SNP rs191531346 constitutes an A to G substitution in less than 1% of all cases. This substitution does not influence the assay since both alleles are identical after bisulfite conversion of the reverse strand to which the assay primers anneal. The influence of SNP rs41213451 is predicted to be negligible as the frequency of the alternative alleles is < 0.05%. The influence of SNP rs3447383 is predicted to be negligible, as it is the last base on the 3' end and is also rare (< 0.3%).

A simulated Septin9 PCR on the human genome (ePCR) was performed using the NCBI database. This simulated PCR reaction demonstrated specificity of the selected Septin9 primer since no amplicon other than the expected Septin9 amplicon was found when using the least restrictive conditions in which a 200bp size deviation, 2 base pair mismatch in each primer, and a 2 base pair gap in each primer was allowed.

A simulated ACTB PCR on the human genome (ePCR), using the NCBI database indicated no second amplicon was found when using the least restrictive conditions in which a 200bp size deviation, 2 base pair mismatch in each primer, and a 2 base pair gap in each primer was allowed.

These studies indicated exclusive and specific primer and probe hybridization.

b. Other Disease States

To determine if there were other conditions or disease states not related to CRC where the Epi proColon assay would report a positive result, a study was done that evaluated other cancer types, medications and other co-morbidities. A total of 386 samples were evaluated (195 cancer of different types, 191 comorbidity/current medications).

22 of the 195 cancer samples were colorectal cancer (CRC) cases, 19 of these 22 cases (86%) were tested positive.

From 173 specimens from symptomatic cancer patients other than colorectal cancer, 72 specimens (42%) were tested positive. While the number of cases is small for several organs, the proportion of positives is dominated by findings in lung cancer (54%) and prostate cancer (25%) specimens.

Among the 191 patients with documented chronic condition/ comorbidity or a current medication, 33 (17 %) were tested positive. For the conditions documented in more than 10 patients, percentages of positive test results range between 0.29 (5/17) for chronic gastritis and 0.05 (1/21) for type II diabetes. Results for conditions documented in less than 10 patients (e.g. diverticulitis, other liver disease), were documented for completeness but final conclusions could not be drawn due to the low number of available samples reflecting the low prevalence in the tested cohort. Current medications were sorted into different categories of drugs have been documented for these patients, but

none of the drug categories have a percent positive test result that was significantly different from the overall proportion of positive test results (p-value 0.73).

To investigate the false positive results seen above, Septin9 positive PCR reactions from non-colorectal cancers plasma and patients with other diseases (co-morbidities) were analyzed for sequence identity and methylation status of Septin9. Seven (7) clinical samples were evaluated by cloned sequencing. For each analyzed reaction the majority of the Septin9 sequences were completely converted and fully methylated. In total 114 of 118 Septin9 sequences were confirmed co-methylated at all 5 CpG positions (97%). These data suggest that the positive result of an Epi proColon test reflects the biological status of the Septin9 gene in the analyzed specimens and is not related to cross-reactivity.

7. Stability Studies

a. Blood/Plasma

Blood was drawn into eight BD Vacutainer K₂EDTA 10 mL tubes for each of the 20 subjects. Blood specimens were collected and processed to plasma within four (4) hours. Four different methods of plasma preparation and storage were implemented. For two methods the plasma was prepared with a single centrifugation to remove cellular material and then was either frozen immediately or kept at a cooled condition (2 – 8°C) for 72 hours prior to freezing. For the other two methods plasma was centrifuged a second time and then either frozen immediately or kept at a cooled condition (2 – 8°C) for 72 hours prior to freezing. For each condition one blood specimen was spiked with HeLa cell line derived DNA (methylated for Septin9), while a second blood aliquot was processed without addition of a DNA spike. After QC of the data, 143 specimens from 18 subjects were available for evaluation. All of the 72 (100%) plasma specimens derived from blood spiked with methylated DNA consistently tested positive for all four storage/processing conditions. Thus, none of the methods of plasma logistics led to loss of the detection of the target DNA by the Epi proColon test. Of the plasma specimens derived from blood without a DNA spike, 68 out of 71 (96%) were negative.

Furthermore, whole blood specimens collected from 16 self-reported healthy subjects in BD Vacutainer K₂EDTA 10 ml tubes spiked with 100 µL CRC plasma into the 10 mL blood sample immediately after blood draw (prior to plasma preparation) as well as unspiked blood specimens were tested.

There was no effect observed on the Epi proColon test result (qualitative and quantitative) of blood storage for an extended period of time, simulating delayed plasma processing in the laboratory (6 hours at room temperature) or overnight storage of whole blood in a refrigerator (24 hours at 2 – 8 °C).

There was no effect observed on the Epi proColon test result (qualitative and quantitative) of plasma being stored at temperatures different from -80°C, simulating shipment of plasma at cooled conditions (72 hours at 2 to 8 °C) or storage of plasma in a standard freezer (14 days at -25 to -15 °C).

There was no effect observed on the Epi proColon test result (qualitative and quantitative) of variations of the centrifugation procedures that simulate changes towards more gentle (e.g. single spin at lower speed + shorter time) or more harsh (double spin at higher speed + longer time) centrifugation conditions.

b. Intermediate Product Stability

To evaluate stopping points in the assay intermediate products (DNA extracted from plasma and purified DNA after DNA extraction and bisulfite conversion) were run under nominal conditions and stored under 5 different time (0, 18, 24 and 72 hrs) and temperature (2 to 8°C, or -25°C to -15°C) configurations. The nominal and test configurations were each evaluated with 6 replicates.

Extracted DNA samples (before bisulfite conversion) held at 2 to 8°C yielded correct results, samples held at -25°C to -15°C failed 5 out of 6 replicates.

Extracted DNA that is bisulfite converted and purified can be held for 24 hrs at 2 to 8°C, or for 72hrs at -25°C to -15°C.

Users will be warned not to store extracted DNA at -25 °C to -15 °C in the instructions for use.

c. Kit Stability: Long Term

Three sample batches were processed with stored kits from three different verification lots for each component of the Epi proColon test. Component kit lots were freely combined for testing at each point in time. Each batch contained eight technical samples, three positive controls (PC) and three negative controls (NC) for testing. The schedule for real time testing covered in total 13 points in time (including time zero). Real time stability has been demonstrated to be at least:

- 13 months for Epi proColon Plasma Quick Kit
- 13 months for Epi proColon Sensitive PCR Kit
- 13 months for Epi proColon Control Kit

d. Transport Stability

Kit stability was also evaluated for different transport stress conditions. After transport stress, kits were evaluated immediately and were placed under normal storage conditions for further testing of long-term effects at pre-defined storage durations.

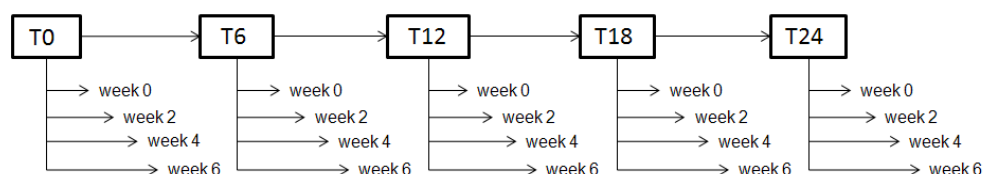
The kits were evaluated under ambient or dry ice transport simulations for 3-5 days depending on the component. In the case of evaluating the Epi proColon Control Kit, the controls themselves were stressed.

Samples evaluated immediately after the simulated conditions indicated that reagents and the controls were stable with all 18 technical samples reporting positive (100%) and both positive and negative controls reported as expected (107/108 valid, 99.1%). Results of long-term storage of the stressed components indicate stress conditions had no influence on product performance.

e. In-use (Open Vial) Stability

For in-use stability testing, an experiment was conducted over 6 weeks for the Epi proColon Plasma Quick kit and Epi proColon Sensitive PCR kit. Kits were opened and reagents that required re-constitution were prepared. The Epi proColon Control kit contains only single use vials, therefore, no in-use stability testing was required.

Within six weeks, four batches were processed with each set of Epi proColon kit components with two weeks between processing of batches. During the study period, partial kits were stored under specified (in-use) storage conditions. Identical experiments were conducted at 6, and 12 months.



Data were analyzed using a logistic regression model with time as the independent parameter.

At time periods T0-T12, all 72 technical samples processed were positive. From T12, it was observed that no loss of performance is detectable when the Epi proColon Plasma Quick Kit (M5-02-001), and Epi proColon Sensitive PCR Kit M5-02-002) were stored as specified and used within 6 weeks.

B. Animal Studies

None.

C. Additional Studies

None.

IX. SUMMARY OF CLINICAL STUDIES

Two studies were performed to demonstrate the clinical performance of the Epi proColon test. The first study compared performance of the Epi proColon test to colonoscopy (Pivotal Study). The second study (Section IX, Summary of Supplemental Clinical Information) compared the performance of the Epi proColon test and the Fecal Immunochemical Test (FIT) to colonoscopy results.

A. Study Design – Pivotal Study

The Pivotal Study for evaluation of the Epi proColon test was designed to validate the intended use of the Epi proColon test by generating data on clinical specimens from an average risk screening population. All subjects donating plasma samples also underwent colonoscopy, so that Epi proColon test results were compared to colonoscopy findings.

The objective of the pivotal study was to evaluate the clinical performance characteristics of the test in terms of sensitivity and specificity for colorectal cancer. As a secondary objective the

test positivity in advanced adenoma (AA), small polyps (SP), and specimen without evidence of disease (NED) were evaluated.

Plasma specimens were collected from a CRC screening eligible population at average risk for CRC under the US Multi-Society Task Force Guideline. Clinical definitions for the specimens from this cohort were as follows:

- CRC - Clinical/surgical diagnosis of invasive colorectal adenocarcinoma detected by optical colonoscopy and confirmed by histology for CRC cases
- Advanced adenomas (AA) including adenomatous polyp(s) equal to or greater than 10 mm, adenomas with a villous component or high grade dysplasia (HGD) as detected by colonoscopy and confirmed by histology.
- Small polyps (SP) – polyps < 10mm and without a villous component or HGD
- No evidence of disease (NED) – no evidence of any of the above

From 32 practices, 22 in the United States, 10 in Germany, 7941 men (45%) and women (55%), average age of 60, were recruited into this sampling study entitled “Prospective Evaluation of Septin9 Performance in CRC Screening (PRESEPT)” between June 2008 and January 2010. Whole blood was collected from each subject, processed to plasma, aliquoted and archived at -80°C for later use in the pivotal study. Specimens from 6857 subjects were available for selection into laboratory analysis.

The Pivotal Study was designed to test all colorectal cancer patients (CRC), all patients with advanced adenomas (AA), and a randomized subset of subjects with small polyps (SP) and no evidence of disease (NED). Selection of subjects within each of the SP and NED classes was done using a stratified random sampling approach, a common statistical method used to achieve a demographic profile, here based on the US 2010 census and the intended use population. As PRESEPT included both US and non-US sites, a stratified random selection of the SP and NED groups was performed to better represent the US population. The samples were selected so that the age distribution matched the US 2010 census. The proportions for the strata defined by ethnic group were chosen such that weights larger than what was enrolled in PRESEPT were placed on non-Caucasian subjects. Although not all the specimens obtained from PRESEPT were tested, all relevant subpopulations were sampled and tested to derive the maximum value from the available sample pool.

1. Inclusion Criteria

Subject inclusion in the study was based on the following:

- Informed Consent provided
- Capable of providing adequate health history
- Age 50 or older at time of colonoscopy (colorectal screening guideline eligible)
- Accessible for blood draw prior to start of bowel preparation for colonoscopy
- First colonoscopy in lifetime

2. Exclusion Criteria

Subject exclusion from the study was based on the following:

- Anorectal bleeding or hematochezia within last 6 months for which patient sought medical attention
- Known iron deficiency anemia in the last 6 months for which patient sought or received medical attention
- Previous history of colorectal polyps or CRC
- High risk for colorectal cancer (2 or more 1^o relatives with CRC; 1 or more 1^o relative(s) < 50 years with CRC; known HNPCC or FAP)

Note: Patients meeting eligibility, but failing inclusion or exclusion criteria were withdrawn from the study prior to blood draw.

Sample exclusion in the study was based on the following:

- Gross hemolysis (bright orange or red color)
- Protocol non-compliant collection, processing, storage or shipping
- Plasma samples with inadequate volume for Septin9 analysis

B. Accountability of PMA Cohort

A total of 1623 subject samples were chosen for evaluation. Randomized sample batches were prepared and the subject identities masked prior to shipment to one of three independent US testing laboratories. Of the subject samples tested, valid results were obtained for 1544. For a total of 79 subjects no results were obtained for the reasons shown in Table 6.

Table 6: Subject Disposition During Testing

	CRC	AA	SP	NED	Total
Total number of unique subjects tested*	50	650	454	469	1623
Numbers of subjects contained in invalid batches indicated by processing controls; no result reported, no repetition possible	5	19	6	13	43
Numbers of subjects with invalid result as indicated by internal control result	0	4	4	5	13
Numbers of subjects excluded due to errors in batch assembly (double measurements)	0	0	2	1	3
Numbers of subjects excluded due to documented technical errors	1	6	7	6	20
Total number of subjects with no result	6	29	19	25	79
Total number of subjects with valid test results **	44	621	435	444	1544

*The subject specimens selected were representative of the demographic factors obtained for study subjects that includes age, gender, race/ethnicity and nationality.

**The total valid test results are obtained by subtracting numbers of row 2, 3, and 4 from number of row 1.

Table 7 provides a break-down of exclusions by number of test results. Numbers of results are greater than the number of unique subjects since some subjects were tested more than once as allowed in the protocol (e.g. process control failures).

Table 7: Sample Disposition During Testing

	CRC	AA	SP	NED	Total
Total number of test results (incl. repeats)	52	738	526	533	1849
Invalid test results reported due to invalid processing batches	7	107	76	76	266
Numbers of results excluded due to errors in batch assembly (double measurements)	0	0	4	2	6
Excluded results due to technical errors	1	6	7	6	20
Excluded results due to invalid internal (ACTB) control	0	4	4	5	13
Total number of invalid test results	8	117	91	89	305
Total valid test results	44	621	435	444	1544

As shown in the last row of Table 7, 1544 subject samples were available for analysis. Among the valid set of available subjects the class sizes were as follows:

- 44 subjects classified as CRC
- 621 subjects classified as advanced adenomas (AA)
- 435 subjects classified as small polyps (SP)
- 444 subjects classified as no evidence of disease (NED)

C. Study Population Demographics and Baseline Parameters

The stratification of the 1544 samples with valid test results by subject demographics of gender, age, race/ethnicity, and country is shown in Table 8. All available CRC and AA subject samples were tested. For SP and NED, equivalent numbers of samples were tested for males and females. Proportions of samples tested in the age groups (50-59; 60-69 and > 69) were based on the US census population. Numbers of samples tested in the minority populations were increased to allow for estimation of test positive rates in these racial/ethnic groups.

Table 8: Demographic Stratification of Evaluable Subjects Tested

Factor	Value	Percent of Total (1544)	Percent (n)			
			CRC (44)	AA (621)	SP (435)	NED (444)
Gender	Female	47	32 (14)	43 (267)	51 (221)	50 (223)

Factor	Value	Percent of Total (1544)	Percent (n)			
			CRC (44)	AA (621)	SP (435)	NED (444)
	Male	53	68 (30)	57 (354)	49 (214)	50 (221)
Age	50 – 59	40	9 (4)	35 (218)	45 (195)	45 (198)
	60 – 69	37	55 (24)	47 (294)	30 (131)	29 (127)
	> 69	23	36 (16)	18 (109)	25 (109)	27 (119)
Race/ Ethnicity	Caucasian	73	89 (39)	85 (527)	66 (288)	63 (278)
	African-American	17	7 (3)	9 (56)	21 (92)	25 (110)
	Others*	10	5 (2)	6 (38)	13 (55)	13 (56)
Country	U.S.A	81	59 (26)	77 (480)	84 (365)	84 (373)
	Germany	19	41 (18)	23 (141)	16 (70)	16 (71)

D. Safety and Effectiveness Results

Clinical performance of the Epi proColon test was evaluated in terms of sensitivity for colorectal cancer and specificity in subjects negative for colorectal cancer as determined by colonoscopy. The results by overall clinical status (CRC and non-CRC) were as shown in Table 9, with the sensitivity and specificity outcome in Table 10.

Table 9: Results of Epi proColon Testing Sample Type

Sample Status	Negative	Positive	Valid Samples
CRC	14	30	44
Non-CRC	1182	318	1500
Total	1196	348	1544

Table 10: Clinical Performance of Epi proColon

Performance Parameter	Point Estimate	Lower 95%	Upper 95%
Sensitivity	68.2 %	53.4 %	80.0 %
Specificity	78.8 %	76.7 %	80.8 %
Specificity* (weighted)	79.1 %	77.0 %	81.4 %
Specificity** (weighted)	80.0%	77.9 %	82.1 %

*weighting according to US census population

**weighting according to PRESEPT patient disposition.

Additionally, the Epi proColon test results were determined for the individual clinical categories including CRC, AA, SP, and NED (Table 11).

Table 11: Epi proColon Test Results by Clinical Category

Sample Status	Negative	Positive	Valid Samples
CRC	14	30	44
AA	487	134	621
SP	348	87	435
NED	347	97	444
Total	1196	348	1544

Predictive values were calculated based on the information provided by the PRESEPT cohort, i.e. the specificity weighted according to the PRESEPT cohort (Table 10) was used and the prevalence of CRC disease was estimated from the set of available 6,857 PRESEPT subjects. The predictive values are provided in Table 12.

Table 12: Predictive Values for VAL0018

Predictive Values	Point Estimate	CI 95%
Positive Predictive Value (PPV)	2.5 %	2.3 – 2.6 %
Negative Predictive Value (NPV)	99.7 %	99.4 – 99.9 %

Logistic regression for non-CRC samples was used for determining whether the diagnosis group, a demographic variable (age, gender, ethnicity, country of origin) or the site of plasma testing had a significant effect on the positive detection fraction (PDF). Based on the likelihood ratio test, only age and ethnicity had an influence on the PDF. The categories for diagnosis, source country, gender, and testing lab were not significantly different from each other. Further evaluation of the data by ethnic groups did not reveal a preponderance of any one non-CRC subtype within the African American subjects, and the increase in PDF values in subjects over 69 years of age has been attributed to age-related increase in methylation as described in the literature.

The variation in the proportions of positive test results that has been observed in non-CRC patients of different demographic sub-category has been investigated with respect to the impact on individuals of these sub-categories. Analysis of the information contained in the Epi proColon test result relevant to the individual by respective diagnostic likelihood ratios within each sub-category revealed, that the test result remains informative to individuals of all demographic sub-categories.

For the CRC data, there was no sub-sampling by Epigenomics and there was no evidence for sensitivity dependence on age-category or race so the observed sample set has been treated as random sample from the target US population.

In the pivotal study 1544 test results were reported as valid out of 1583 test results. This provided an estimate for the proportion of plasma samples suited for analysis with the Epi proColon test of 97.5 %. This finding is in line with similar results from the verification studies utilizing clinical samples.

Overall, in this cohort, the Epi proColon test shows a sensitivity of 68% which is within the expected range and within the defined specifications. In the pivotal study, cancer detection was measured against the standard of optical colonoscopy, and although we did not compare directly with immunochemical fecal occult blood tests in the Pivotal Study, the sensitivity estimate of 68% falls within the reported ranges for this non-invasive test, meeting the specified criteria in the DIR. An additional study, a direct comparison to FIT was performed in which this observation was corroborated with a reported sensitivity estimate of 72%.

In the non-cancer subgroups tested an adjusted specificity of 80% was observed for the Epi proColon test, which is similar to the specificity observed for Epi proColon in the direct comparison to FIT. The consequence of a positive result for the Epi proColon test is that these individuals will be recommended to undergo a follow up diagnostic procedure such as colonoscopy. In the case of a positive Epi proColon test with a positive follow-up colonoscopy, the patient's physician will proceed according to guidelines for treatment. In the case of a positive Epi proColon test with a negative follow-up colonoscopy, the situation would be similar to a false positive fecal occult blood test. A recent article by Allard et al. propose that when there is insufficient evidence to recommend for or against further diagnostic work-up for false positive FOBT (esophageal/gastro) follow-up should be based on the individual patient and the physician's clinical judgment (Allard 2010).

In summary, Epi proColon is not inferior to current non-invasive tests with respect to sensitivity. Furthermore, while the test showed lower specificity results than had been required by the Design Input Requirement, given that the reflex to a positive test would be colonoscopy, a currently approved screening methodology, the Epi proColon test does not create an additional safety burden, and presents a screening method that should have a higher acceptance rate within the intended use population.

X. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

In performing the Pivotal Study based on the PRESEPT prospective clinical trial, Epigenomics demonstrated that the Epi proColon test detected 68% of cancers in an average risk population as verified by optical colonoscopy. The protocol for patient enrollment was designed for comparison to the reference standard (optical colonoscopy) and did not include other approved screening modalities (e.g. detection of Fecal Occult Blood). Given that the Epi proColon test presents a new non-invasive screening modality, the company sponsored a second trial to compare performance of their test to a commercially available and broadly used immunochemical Fecal Occult Blood test (OC FIT-CHEK® Polymedco), using colonoscopy as the reference standard.

The study for the comparison of Epi proColon to FIT was designed to collect matched blood and stool specimens and clinical data from screening guideline-eligible subjects. The study had two collection arms:

- Group A patients were those found to have invasive colorectal cancer (CRC) or a high suspicion of invasive CRC identified during in a screening colonoscopy, i.e. AJCC/UICC stages I, II, III, and IV, and for whom collection of blood and stool occurred after colonoscopy but prior to surgery or intervention.
- Group B subjects were prospectively enrolled and provided blood and stool samples prior to screening colonoscopy

The primary objective of the investigation was to compare the clinical performance in terms of test positivity of the Epi proColon test to the OC FIT-CHEK® (Polymedco, Inc) test. The Group A arm of the study provided a sufficient sample size of cases to perform a sensitivity comparison. The Group B arm of the study provided a sufficient sample size to perform a specificity comparison.

Blood samples were acquired from each subject, processed to plasma and aliquoted. These aliquots were shipped frozen to a central repository and archived at -80°C for later testing. Stool samples were collected by the subjects using supplied kits and shipped directly to the testing laboratory.

Clinical diagnostic categories of the subjects enrolled include colorectal cancer (CRC), advanced adenoma (AA), small polyps (SP) and no evidence of disease (NED). A total of 337 subjects were enrolled in the study. A total of 36 subjects were excluded from testing due to failure to meet inclusion/exclusion criteria. Of the remaining 301 subjects, 301 plasma samples and 290 stool samples were collected according to SPR 0022 and were evaluable. Of these, there were 101 CRC, 29 AA, 77 SP and 94 NED. Fecal samples were not available from 11 subjects (4 CRC, 2 AA, 2 SP and 3 NED).

Both Epi proColon testing and OC FIT-CHEK testing were performed at an independent clinical laboratory in the US.

The data presented in Tables 12-15 is a comparison of the Epi proColon test results and FIT test results versus colonoscopy. In this case the subjects/samples evaluated by all three methods are the same (or paired). Comparison of the Epi proColon results to colonoscopy can be found in Table 12 with the corresponding sensitivity and specificity results in Table 13.

12: Epi proColon vs. Colonoscopy Results

Candidate Method	Diagnostic Accuracy Criteria		
Epi proColon	CRC Reference Standard		
	Positive	Negative	Total
Positive	74	37	111
Negative	27	163	190
Total	101	200	301

Table 13: Epi proColon Sensitivity and Specificity

Epi proColon		95% CI	
		Lower	Upper
Sensitivity	73.3%	63.9	80.9
Specificity	81.5%	75.5	86.3

Comparison of the FIT results to colonoscopy can be found in Table 14 with the corresponding sensitivity and specificity results in Table 15.

Table 14: FIT vs. Colonoscopy Results

Alternative Method	Diagnostic Accuracy Criteria		
FIT	CRC reference standard		
	Positive	Negative	Total
Positive	66	5	71
Negative	31	188	219
Total	97	193	290

Table 15: FIT Sensitivity and Specificity

FIT		95% CI	
		Lower	Upper
Sensitivity	68.0%	58.2	76.5
Specificity	97.4%	94.1	98.9

A three-way summary of the Epi proColon test results, FIT test results and colonoscopy results can be found in Table 16.

Table 16: Three-Way Comparison of the Epi proColon and FIT Tests, and Colonoscopy (paired samples)

Diagnostic Accuracy Criteria: Standard Colonoscopy						
Colorectal Cancer				Non-Colorectal Cancer AA, SP, NED		
	Epi proColon Positive	Epi proColon Negative	Total	Epi proColon Positive	Epi proColon Negative	Total
FIT Positive	50	16	66	1	4	5
FIT Negative	20	11	31	36	152	188
Total	70	27	97	37	156	193

The observed sensitivity for CRC on paired samples was 4.2% higher (Epi proColon: 72.2%, FIT: 68.0%) for the Epi proColon test. The 95% confidence interval of the sensitivity difference (-16.2%; 8.1%) was strictly below the non-inferiority margin of 10% pre-set in the protocol.

Therefore, the sensitivity of the Epi proColon test is statistically non-inferior to the FIT test. For specificity, the difference between tests was 16.6% (Epi proColon: 80.8%, FIT: 97.4%) in favor of the FIT with a 95% confidence limit (10.6%; 22.9%) around the estimate. This result does not demonstrate non-inferiority for specificity when compared to the non-inferiority margin of 20% pre-set in the protocol.

When the two methods are compared via diagnostic likelihood ratios, the relation between the positive likelihood ratios is in advantage for FIT, while the negative likelihood ratios are virtually identical. These relations translate one-to-one to the comparison of positive and negative predictive values.

Further analysis of the study did not reveal any difference between tests with regard to the detection of very early (stages 0, I) and early cancer (stage II) providing evidence that Epi proColon is not inferior to FIT with respect to detection of early cancers. Furthermore, there were no relevant differences in tumor detection with regard to tumor location, age or gender. Also, with regard to ethnicity, there were no major differences in tumor detection rates with the exception of the Hispanic population where FIT had an unexpectedly low detection rate (47%). There were no significant differences in tumor detection with regard to tumor location, though the Epi proColon test detected CRC slightly better than FIT on the right side of the colon while FIT had slightly higher detection than Epi proColon on the left side.

Both tests failed to show any relevant detection of advanced adenomas (FIT: 2/27; Epi proColon: 4/29) or small polyps (FIT: 1/75; Epi proColon: 11/77) in this study.

Based on the results obtained for sensitivity, both tests will identify a similar high number of CRC patients per patients tested, even though not necessarily the same individuals. Therefore, both methods support the primary objective of a screening modality, i.e. identification of diseased subjects at a curable stage. The risk to the patient posed by a false negative test result described by the negative predictive value is very comparable between both tests. Both tests perform equally well in confirming the absence of disease.

The comparison data strongly support the efficacy of Epi proColon as a new screening modality. In practice, if screening participation improves as a result of the use of a blood based test, given a non-inferior cancer detection rate, additional cancer patients should be detected in the population with the Epi proColon test. From a safety perspective, the consequence of a higher false positive rate, or lower specificity, will be that more patients are recommended for colonoscopy follow up. Given that screening by colonoscopy is a currently approved and recommended screening modality, though having some risk of adverse events, we believe that this is an acceptable safety risk.

XI. CONCLUSIONS DRAWN FROM STUDIES

Two studies were performed in support of the Epi proColon PMA. The Pivotal Study compared the performance of the Epi proColon test to colonoscopy in the average risk colorectal cancer screening population. The second study compared the performance of the Epi proColon test to an FDA-cleared FOB assay.

In the Pivotal Study cohort, the Epi proColon test had a sensitivity of 68%. In this study, cancer detection was measured against the standard of optical colonoscopy. The reported sensitivity estimate of 68% for Epi proColon falls within the reported ranges for FOB assays. In an additional study, a direct comparison to FIT was performed in which this observation was corroborated with a reported sensitivity estimate for the Epi proColon test of 70%.

In the non-cancer subgroups tested in the Pivotal Study we observed a specificity of 79%. A similar observed specificity of 81% was found in the direct comparison to FIT study (SPR0022) for both Epi proColon and FIT.

A. Risk/benefit Analysis

The data support the use of the Epi proColon test as a new CRC screening modality, given that the cancer detection rate is similar to that of FIT, the current non-invasive alternative, the use of the Epi proColon test would not result in an increase in missed cancers in a CRC screening program. The negative predictive value of both test methods was equivalent. Therefore the test does not increase risk to patients. The probable benefit to patients is the expected increase in compliance/adherence to CRC screening which could result in an overall improvement in early CRC detection rates.

B. Safety

The risks of the device are based on performance demonstrated in nonclinical and clinical studies. False positive and negative results are discussed in Section VII Potential Adverse Effects of the Device on Health. The Epi proColon test involves the removal of a blood sample from an individual for the purpose of testing. As a common minimally invasive method, the test presents no more of a safety hazard to an individual being tested than other tests where blood is drawn. There were no adverse effects of the device reported during either clinical study conducted.

Subjects with a positive Epi proColon test will be recommended to undergo colonoscopy as a follow-up diagnostic procedure (similar to FOB tests). In the case of a positive Epi proColon test with a positive follow-up colonoscopy, the patient's physician will proceed according to guidelines for management and treatment of colorectal cancer. In the case of a positive Epi proColon test with a negative follow-up colonoscopy, the situation would be similar to follow up for false positive fecal tests. A recent article by Allard et al. proposes that when there is insufficient evidence to recommend for or against further diagnostic work-up for false positive FOBT (e.g. upper endoscopy) follow-up should be based on the individual patient and the physician's clinical judgment (Allard 2010).

The negative predictive value of the Epi proColon test is equivalent to other non-invasive screening methods (FIT), thus, not posing an increased safety risk to patients.

C. Effectiveness

In summary, the Epi proColon test is an effective method for CRC screening. The test is not inferior to current non-invasive tests with respect to sensitivity. Furthermore, while the test showed lower specificity results than had been expected, given that the reflex to a positive test would be colonoscopy, a currently approved and recommended screening methodology, the

Epi proColon test does not create a safety burden, and presents an alternative screening method that may lead to an overall higher participation rate for colorectal screening within the intended use population.

XII. PANEL RECOMMENDATION

XIII. CDRH DECISION

XIV. APPROVAL SPECIFICATIONS

XV. REFERENCES

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